Characterization of Two *O*-Methyltransferase-like Genes in Barley and Maize*

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Abstract

Tricin (3′,5′-dimethoxy-5,7,4′-trihydroxyflavone) is a characteristic flavone constituent of cereal grain plants that is credited for a variety of potential health benefits to humans. We have characterized the flavone-specific *O*-methyltransferase genes of barley (*Hordem vulgare* L., Gramineae), *HvOMT1,* and maize (*Zea mays* L., Gramineae), *ZmOMT1*, whose gene products use the flavone tricetin (5,7,3′,4′,5′-pentahydroxyflavone) as the preferred substrate and give rise to its 3′,5′-dimethyl derivative, tricin, as the major product. The fact that homologous enzymes catalyze the same reaction also in wheat and rice suggests the existence in cereal grain plants of a flavone-specific *O*-methyltransferase multigene family. The natural occurrence of tricin in most monocot species implies the widespread occurrence of this gene family. The pharmacological significance of tricin as a naturally occurring constituent with a potential use as a nutraceutical, and the application of metabolic engineering methods to obtain tricin-enriched cereal grain products, are discussed.

Keywords: Cereal crop plants, flavones, gene family, *O*-methyltransferase, phylogeny, substrate preference, tricin.

Introduction

One of the unique features of plants is their ability to synthesize and accumulate a bewildering array of organic compounds, collectively known as secondary metabolites. Of these, flavonoid compounds, which are formed via the phenylpropanoid and polyketide pathways, constitute one of the major classes of plant natural products. They are considered an important part of the human diet and act as active principles of many medicinal plants. Several biological and/or pharmacological activities have been attributed to flavonoids as antioxidants, radical scavengers, and antimutagenic, antiviral, antiinflammatory, and anticarcinogenic agents, to mention a few (Bohm, 1998 and refs. therein). According to the oxidation level of the heterocyclic ring, flavonoids are subdivided in to several groups that include the chalcones, flavones, isoflavones, flavonols, and anthocyanidins. Later steps in flavonoid biosynthesis are catalyzed by a number of substrate-specific, position-oriented enzyme reactions, such as hydroxylation, glycosylation, acylation, methylation, and prenylation (Ibrahim & Anzellotti, 2003).

O-Methylation of flavonoids is catalyzed by an extensive family of *O*-methyltransferases (OMTs) and involves the transfer of the methyl group of *S*-adenosyl-L-methione (AdoMet) to a specific hydorxyl group of a suitable flavonoid acceptor, with the concomitant formation of its corresponding methyl ether derivative and *S*-adenosyl-L-homocysteine (AdoHcy) as products. It reduces the reactivity/mutagenicity of phenolic hydroxyl groups (Zhu et al., 1994) and increases the lipophilicity of flavonoids and, hence, their antimicrobial activity. A number of flavonoid OMT genes have been cloned and their gene products biochemically characterized (Ibrahim et al., 1998; Ibrahim & Muzac, 2000; Lam et al., 2007 and refs. therein).
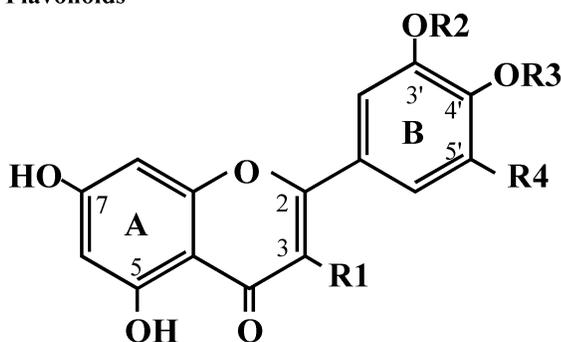
One of the typical flavonoid constituents of monocotyledonous species is the flavone, tricin (Fig. 1). It was first isolated from wheat (Anderson, 1932), and has been detected

Flavonoids

R1	R2	R3	R4	
H	H	H	OH	Tricetin
H	Me	H	OH	Selgin
H	Me	H	OMe	Tricin
H	H	H	H	Luteolin
H	Me	H	H	Chrysoeriol
H	H	H	H; (2,3 = H,H)	Eriodictyol
OH	H	H	H	Quercetin
OH	H	H	H; (2,3 = H,H)	Taxifolin
OH	H	H	OH	Myricetin

Hydroxycinnamic acids
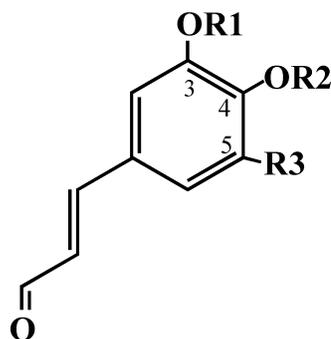
R1	R2	R3	
H	H	H	Caffeic
Me	H	H	Ferulic
Me	H	OH	5-Hydroxyferulic
Me	H	OMe	Sinapic

Figure 1. Structural formulae of the flavonoids and hydroxycinnamic acids used in this study.

in 91% of 118 species tested in the Graminae and in almost 50% of the species examined from both the Cyperaceae and Palmae (Harborne, 1975). We have recently isolated and characterized the flavone-specific OMTs of wheat (Zhou et al., 2006a) and rice (Zhou et al., 2006b), both of which use the pentahydroxyflavone tricetin (Fig. 1) as the preferred substrate and produce tricin as the major enzyme reaction product. Our interest in the OMTs of cereal grain plants prompted us to investigate other flavonoid OMT genes in order to expand the repertoire of this gene family.

In this work, we report on the biochemical characterization of two OMT-like genes from barley (*HvOMT1*) and maize (*ZmOMT1*), whose gene products use the flavones tricetin and luteolin (Fig. 1) as the preferred substrates. The fact that the maize OMT gene is identical in its amino acid sequence to another maize OMT, previously reported as a caffeic acid OMT (COMT) gene (Collazo et al., 1992), prompted us to investigate the substrate preference of ZmOMT1 and its phylogenetic relationship to other known cereal grain plant OMTs. The results of this

study strongly suggest the existence of a flavone-specific OMT gene family in cereal grain plants that may extend to other monocot species and calls for the reexamination and reannotation of the previously reported COMT genes.

Materials and Methods

Chemicals

Most of the flavonoid compounds used in this study were from our laboratory collection, except tricetin, which was purchased from Indofine Chemical Company (Hillsborough, NJ, USA). The methylated tricetin derivatives, which are not available commercially, were synthesized by standard methods, and their purity and identity were verified by UV and ^{1}H NMR spectroscopic methods. S-Adenosyl-L-[^{14}C]methionine (AdoMet; 55mCi/mmol) and S-adenosyl-L-[^{3}H]methionine (80 Ci/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA), and unlabeled AdoMet from Sigma (Oakville, ON, Canada). Protein quantification reagents and 40% acrylamide/bis solution (37.5:1) were purchased from Bio-Rad (Mississauga, ON, Canada). Unless otherwise specified, all other chemicals were of analytical grade.

BLAST search and sequence analysis of barley *HvOMT1* and maize *ZmOMT1*

The amino acid sequence of wheat (*Triticum aestivum* L., Gramineae) TaOMT2, GeneBank accession no. DQ223971 (Zhou et al., 2006a), was used to BLAST search the BarleyBase cDNA database (http://www.plexdb.org). The putative barley (*Hordeum vulgare* L., Gramineae) *HvOMT1* cDNA clone (BarleyBase no. 002N02) and maize (*Zea mays* L., Gramineae) *ZmOMT1* cDNA clone (BarleyBase no. 0040C07), which exhibited the highest identity scores, were obtained from the Arizona Genomics Institute Resource Center (Tucson, AZ, USA). Both cDNA clones were sequenced by Génome Québec Innovation Centre (McGill University, Montréal, QC, Canada) before subcloning. The GenBank accession numbers of the barley and maize cDNA clones are EF586876 and EF586877, respectively.

Expression and purification of HvOMT1 and ZmOMT1 in *E. coli*

After sequence confirmation, the open-reading frames (ORFs) of *HvOMT1* and *ZmOMT1* cDNAs were amplified and subcloned into the expression vector pET200/D-TOPO for *in vitro* protein expression. The primers used for PCRs were as follows: for HvOMT1F: 5′-CACCATGGGGTCCATCGCCGCCGGCGCC-3′; HvOMT1B: 5′-CTACTTGGTGAACTCTAGGGCCCA-3′; and for ZmOMT1F: 5′-CACCATGGGCTCCACCGCC-3′; ZmOMT1B: 5′-TCACTTGATGAACTCGATGGCCCA-3′. To reinforce the reliability of cDNA ORF sequences,

AccuPrime*Pfx* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) was used in the PCR reaction following the manufacturer's manual. PCR was performed on PTC DNA 200 Thermal Cycler (GMI, Ramsey, MI, USA): 94°C (2 min), followed by 35 cycles at 94°C (30 s), 52°C (30 s), 68°C (1 min), and a further extension at 68°C (8 min). The PCR product was resolved by electrophoresis on a 1% agarose gel. After electrophoresis, the expected DNA band was recovered with DNA Gel Extraction Kit (Qiagen, Mississauga, ON, Canada), and cloned into pET200/D-TOPO expression vector (Invitrogen). The sequence and orientation of cDNA ORFs in the expression vector were confirmed by sequencing before chemically transformed into *E. coli* BL21 (DE3) cells (EMD, Darmstadt, Germany) for protein production.

In order to characterize the expressed protein, a single colony was incubated in 5 mL of Luria-Bertani (LB) medium containing 100 μg/mL kanamycin and grown overnight at 37°C. The innoculum was then added to 100 mL of LB medium containing kanamycin (100 μg/mL) and grown at 37°C until the OD_{600} reached 0.8, followed by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM. The culture was incubated at 37°C for an additional 4 h, then harvested by centrifugation (8000 $\times g$, 8 min) and stored at -80°C until further protein purification.

For protein extraction, the cell pellet from 100 mL culture was resuspended in a mixture of 4 mL Bugbuster Protein Extraction Reagent, 100 U Benzonase Nuclease, and 10 KU rLysozyme (EMD, Darmstadt, Germany) and agitated at 500 rpm on an orbital shaker for 20 min at ambient temperature. The suspension was centrifuged at 15,000 $\times g$ for 20 min, and the supernatant was used for immobilized metal affinity chromatography (Ni-NTA; Qiagen) following the manufacturer's instructions. The affinity-purified protein fraction was passed through a prepacked PD10 column for desalting. SDS-PAGE was used to check purity of the protein preparations, and the highly purified fraction was stored at 4°C until assayed.

SDS-PAGE and protein determination

The quality of purified protein was monitored by SDS-PAGE analysis according to a standard method (Laemmli, 1970) using Benchmarker (Invitrogen) as a molecular weight ladder. After electrophoresis, proteins were stained with Coomassie Brilliant Blue (R-250). Protein concentrations were estimated using the Bio-Rad protein assay and bovine serum albumin as the standard protein.

OMT enzyme assay and identification of reaction products

The standard OMT assays were performed as previously described (Zhou et al., 2006a) using 50 μM of the phenolic compounds, 50 μM AdoMet, containing 25 nCi of the

[14]C-label, and 0.1 to 2.0 μg of the affinity-purified recombinant proteins. The reaction products from several radiolabeled enzyme assays were combined, lyophilized, then redissolved in 20 μL of MeOH for chromatography on TLC plates (Silica gel 60 F254: Merck, Darmstadt, Germany) in toluene-dioxane-acetic acid (18:5:1, v/v/v) as the solvent system. Control assays were also performed in the absence of substrate or with boiled enzyme for background correction, and all assays were conducted in duplicate. The identity of methylated reaction products was confirmed by co-chromatography with reference compounds, visualized under UV light, and autoradiographed on x-ray film. Semipreparative incubations were also carried out using nonlabeled AdoMet, and the methylated reaction products were prepared for HPLC. HPLC analysis was carried out with a Millennium HPLC System (Waters, Milford, MA, USA), using a Waters YMC-PackPro C18 column (150 $\times$ 4.6 mm I.D., S-5 μM), with a linear gradient consisting of 40–90% MeOH in 1% acetic acid for 30 min, at a flow rate of 1.0 mL/min. This gradient was maintained for a further 10 min before returning to the initial conditions. Identity of the reaction products was confirmed by comparing their retention times (R_t) and their UV absorption maxima with those of the reference compounds.

Kinetic analysis of methylation

Kinetic analyses were performed using 0.1 μg of the affinity-purified OMT proteins with a saturating concentration of AdoMet (50 μM), containing 25 nCi of radioactivity, and varied concentrations (5 to 50 μM) of the flavonoid substrates according to the method previously described (Huang et al., 2004). Assays were performed in triplicate and were repeated twice. Lineweaver-Burk plots (Segel, 1975) were applied for the determination of K_m and V_{max} values.

Phylogenetic analysis

In order to study the phylogenetic relationships among HvOMT1, ZmOMT1, and other monocot plant OMTs, a number of closely related OMT amino acid sequences were aligned with these two OMTs using Clustal W (Thompson et al., 1994). Phylogenetic analysis was conducted using MEGA3 (Kumar et al., 2004). The neighbor-joining method was employed to generate the tree, and bootstrap analysis (100 replicates) was applied to evaluate the reliability of the tree. *Populus tremuloides* (PtCOMT) was used as an outgroup to root the tree.

Results and Discussion

We recently characterized a flavone-specific OMT gene, *TaOMT2*, from a wheat cDNA database, whose gene product uses the flavone tricetin as the preferred substrate (Zhou

et al., 2006a). A BLAST search of the GenBank using the wheat clone as a template resulted in a number of OMT-like cDNA clones. Of these, both maize and barley gene products exhibited respectively 79% and 96% identities to TaOMT2 (Table 1). The maize clone was 100% identical in its amino acid sequence to another maize clone (GenBank accession no. M73235), previously reported as a COMT gene (Collazo et al., 1992). This prompted us to investigate the phylogenetic relationship among a number of monocot OMTs that were previously annotated based on their sequence homology to the maize gene.

Phylogenetic analysis of some monocot OMTs

Phylogenetic analysis of the OMTs shown in Table 1 shows the distinct separation between the monocot and dicot species. The former group is further subdivided into two major clades; one clade includes the maize, sorghum, sugarcane, and rice OMTs, and the other clade combines the barley, wheat, and the two grass enzymes; all of which are supported by high bootstrap values (Fig. 2). The fact that at least two to three OMTs in each clade were characterized as flavone-specific OMTs (see below) supports the view that a flavone-specific OMT gene family may in fact be widespread in cereal grain plants, which may possibly extend to other monocot species. More monocot species OMTs need to be investigated and those previously annotated as COMTs need to be reexamined in order to validate this view.

Characterization of barley and maize OMT genes

The full-length of *HvOMT1* cDNA is 1360 bp and contains an ORF of 1071 bp that encodes a 356-amino-acid polypeptide, with a calculated M_r of 38.7 kDa and a theoritical pI of 5.64. The full-length of *ZmOMT1* cDNA consists of 1482 bp and an ORF of 1095 bp that encodes a 364-amino-acid polypeptide with a calculated M_r of 39.6 kDa and a theoritical pI of 5.48. The apparent molecular masses of both proteins are representative of class II OMTs (Joshi et al., 1998), being similar to those reported for wheat and rice plants (Zhou et al., 2006a,b). When assayed in the presence of 50 mM phosphate buffer, both OMTs exhibited a pH optimum of 7.5, and required no Mg^{2+} for activity (data not shown). Both proteins are stable when stored in the assay buffer containing 10% (v/v) glycerol for 3–4 weeks at 4°C with no appreciable loss of catalytic activity.

Identification of the substrate preferences of barley and maize OMTs

The recombinant proteins of both maize (ZmOMT1) and barley (HvOMT1) were affinity purified (Fig. 3A) and assayed against a number of flavonoid compounds and

Table 1.   Characteristics of some monocotyledonous *O*-methyltransferase genes.

Plant species	GenBank accession no.	Identity similarity to TaOMT2		Preferred substrate(s)	Reference
Triticum aestivum (TaOMT2)	DQ223971	100	100	Flavones	Zhou et al., 2006a
Triticum aestivum (TaCOMT1)	AAP23942	93	96	Flavones	Jang et al., 2005, and this work
Oryza sativa (OsOMT1)	DQ530257	79	85	Flavones	Zhou et al., 2006b
Oryza sativa (OsCOMT1)	XM_480185	79	85	Eriodictyol> caffeic acid	Lin et al., 2006
Zea mays (COMT)	M73235	79	86	Caffeic acid	Collazo et al., 1992
Zea mays (ZmOMT1)	DR811764	79	86	Flavones	This work
Hordeum vulgare (HvOMT1)	BI956358	96	97	Flavones	This work
Sorghum bicolor (COMT)	AY217766	78	85	n.d.[a]	Bout et al., 2003
Saccharum officinarum (COMT)	AJ231133	80	87	n.d[a]	Selman-Housein et al., 1999
Lolium perenne (LpCOMT1)	AF010291	88	93	Caffeic acid	McAlister et al., 1998
Festuca arundinacea (COMT)	AF153825	90	94	n.d.[a]	GenBank

[a]Not determined; characterized by sequence homology to *Zea mays* COMT.

hydroxycinnamic acids as methyl acceptors. The results obtained indicate that both OMTs exhibit an almost similar preference for the flavones luteolin and tricetin, as well as the flavonol quercetin, albeit to a lesser extent (Table 2). However, kinetic analyses of maize and barley OMTs (Table 3) indicate a respectively four-fold and two-fold higher affinity for tricetin over luteolin, as well as an approximately two- to three-fold higher turnover of tricetin compared with luteolin. In contrast with TaOMT2, which catalyzed the sequential methylation of tricetin to its 3′-methyl-(selgin), 3′,5′-dimethyl (tricin), and 3′,4′,5′-trimethyl ether derivatives (Zhou et al., 2006a), both ZmOMT1 and HvOMT1 methylate tricetin to selgin and tricin, with the latter being the major enzyme reaction product (Fig. 3B, C). Furthermore, luteolin was methylated to its 3′-methyl ether derivative, chrysoeriol, as a major product and a minor dimethylated product that co-chromatographed with an authentic sample of luteolin 3′,4′-dimethyl ether, but not the 7,3′-isomer, both of which have distinctly different rentention times on HPLC (data not shown). The enzyme reaction products of tricetin were characterized by co-chromatography with reference compounds and autoradiography (Fig. 3B) as well as HPLC analysis (Fig. 3C). In contrast with flavones and flavonols, the dihydroflavone eriodyctiol and the dihydroflavonol taxifolin (Fig. 1) were comparatively poor methyl acceptors (Table 2), suggesting the requirement of a 2,3-double bond in the flavonoid heterocyclic ring for catalytic activity.

On the other hand, the relative enzyme activities of ZmOMT1 and HvOMT1 toward 5-hydroxyferulic (5HF) acid, a better methyl acceptor by COMT than caffeic acid (Parvathi et al., 2001), were 61% and < 20%, respectively, compared with tricetin (Table 2). These results indicate that both ZmOMT1 and HvOMT1 are flavone-specific OMTs that exhibit expressed preference for tricetin > luteolin as the methyl acceptor molecules, and none of which is a COMT as was earlier claimed for the maize COMT. In fact, characterization of the latter enzyme was based on assays of the "bacterial-pellet" crude extracts with 2 mM caffeic acid as the only substrate used, incubated for 2 h, and no kinetic or turnover values were reported (Collazo et al., 1992), which calls for reexamination of its characterization.

The flavone-specific OMT gene family in cereal grain species

Table 1 summarizes the characteristics of some of the monocot plant OMT genes reported in the GenBank, including eight cereal grain plant OMTs. We have recently characterized the flavone-specific OMTs in wheat (Zhou et al., 2006a) and rice (Zhou et al., 2006b) as well as those of barley and maize (this work). A putative wheat TaCOMT1, with 93% identity and 96% similarity to TaOMT2 (Jang et al., 2005), was recently shown to use tricetin (100%) and luteolin (72%), compared with 17% for 5HF acid
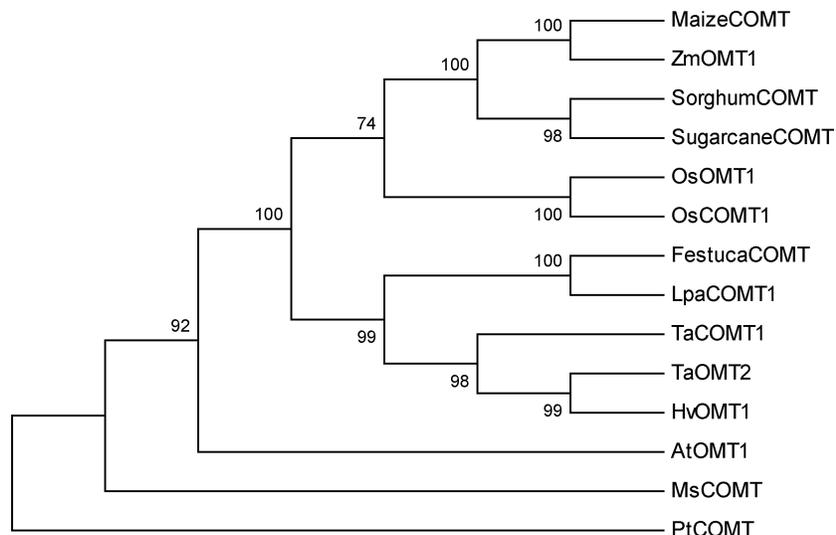
Figure 2. Neighbor-joining tree of some monocot plant OMTs reported in this study. Monocot OMT amino acid sequences were aligned with wheat TaOMT1 using Clustal W (Thompson et al., 1994). Phylogenetic analysis was conducted using MEGA3 (Kumar et al., 2004). The neighbor-joining method was employed to generate the tree, and bootstrap analysis (100 replicates) was applied to evaluate the reliability of the tree. Numbers at branching points are the bootstrap values indicating the confidence level for each branch. The amino acid sequences of three dicot plant OMTs were obtained from GenBank with accession numbers: *Arabidopsis thaliana* flavonol 3'-OMT (AtOMT1, U16794), *Medicago sativa* COMT (MsCOMT, AAB46623), and *Populus tremuloides* COMT (PtCOMT, X62096); the latter served as an outgroup. See Table 1 and the text for details on each OMT.
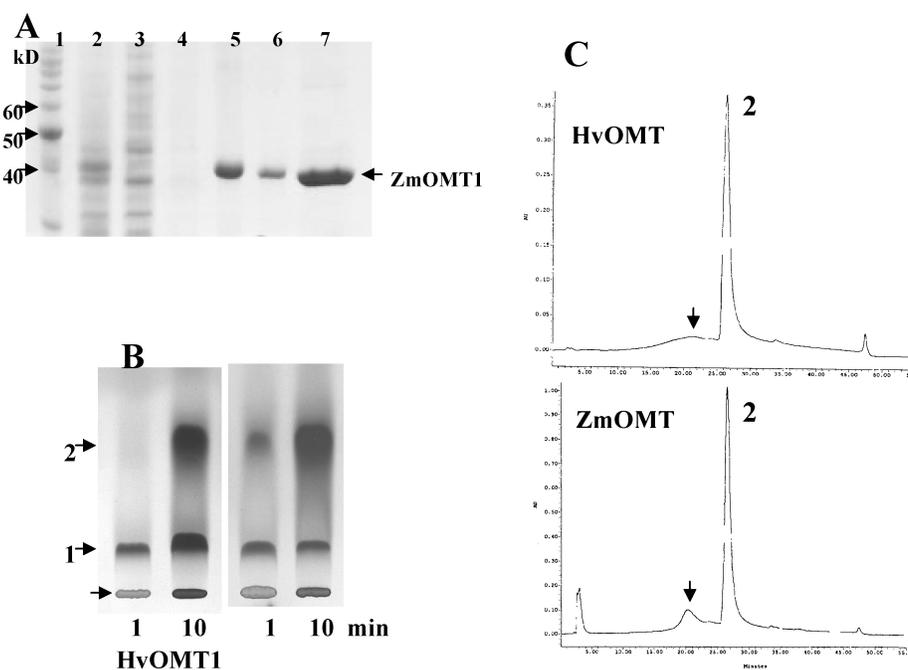

Figure 3. Affinity purification of the recombinant HvOMT1 (not shown) and ZmOMT1 and characterization of their enzyme reaction products using tricetin as the substrate. (A, SDS-PAGE profiles of the recombinant ZmOMT1 protein during affinity purification. 1, Mr ladder of standard proteins; 2, *E. coli* lysate (10 μg); 3, flow-through (10 μg); 4, wash; 5, Ni-eluate (2 μg); 6, desalted protein (0.2 μg); 7, concentrated protein (10 μg). (B) Autoradiogram of the chromatographed enzyme reaction products formed by the HvOMT1 and ZmOMT1 recombinant proteins, with tricetin as the substrate, after 1 and 10 min incubations; 1 and 2 refer to the 3'-monomethyl ether (selgin) and 3',5'-dimethyl ether (tricin) derivatives, respectively. (C), HPLC separation of the reaction products catalyzed by HvOMT1 (top) and ZmOMT1 (bottom); the peak under the arrow represents the residual substrate; 2, tricin. Characterization of the reaction products in (B) and (C) was carried out as described in the "Materials and Methods" section.

Table 2. Substrate preferences of maize and barley *O*-methyltransferases.[a]

| Substrate[b] | Relative activity (%)[c] | |
	ZmOMT1	HvOMT1
Luteolin	100	100
Tricetin	95	88.9
Quercetin	77	80
5-Hydroxyferulic acid	60.8	<20
Eriodictyol	<20	<20
Taxifolin	<20	<10

[a]Enzyme assays were conducted as with the affinity-purified proteins as described in the "Materials and Methods" section.
[b]Names and structures are shown in Fig. 1.
[c]100% relative activity represents 0.39 and 0.45 pkat. mg^{-1} of the recombinant ZmOMT1 and HvOMT1 proteins, respectively.

(unpublished data). A rice OMT, designated OsCOMT1, was recently reported (Lin et al., 2006) to exhibit relative activity preferences for dihydroxy flavonoids, such as eriodictyol (130%), luteolin (88%), myricetin (75%), and quercetin (71%), compared with caffeic acid (100%), although no kinetic data were provided to indicate the real substrate preference of this enzyme. However, the latter authors predicted that tricetin (the flavone analogue of myricetin) and its monomethyl ether selgin, although not tested as substrates, would be good methyl acceptors of OsCOMT1 for the biosynthesis of tricin, which occurs naturally in rice plants (Kuwatska & Oshima, 1961). The fact that OsCOMT1 exhibits 100% sequence identity to the recently characterized flavone-specific OsOMT1 (Zhou et al., 2006b) strongly suggests that the former enzyme is a flavone OMT, especially if kinetic analysis of the OMT is investigated for its preferred substrates.

Characterization of the two putative COMTs from sorghum (Bout & Vermerris, 2003) and sugarcane (Selman-Housein et al., 1999) was solely based on sequence homology with the maize COMT, which itself was insufficiently characterized (Collazo et al., 1992). The ryegrass COMT1

Table 3. Kinetic parameters of maize and barley *O*-methyltransferases for their best substrates.[a]

| Substrate | ZmOMT1 | | HvOMT1 | |
	Tricetin	Luteolin	Tricetin	Luteolin
K_m (μM)	4.17	15.92	2.48	6.18
V_{max} (pkat. mg^{-1})	3.14	22.47	5.28	6.86
V_{max}/K_m	3.15	1.41	2.13	1.11

[a]The affinity-purified recombinant proteins (0.2 μg) were incubated with various concentrations (5 to 80 μM) of the indicated substrates and saturated concentrations of AdoMet containing 25 nCi of [^{14}C]AdoMet for 20 min at 30°C. The activities in reaction products and kinetic analyses were performed as described in the "Materials and Methods" section.

(McAlister et al., 1998) was tested only against caffeic acid, to the exclusion of any flavonoid substrate. Although the ryegrass, sorghum, and sugarcane OMTs exhibit high identity (78% to 88%) and similarity (85% to 93%) to the wheat TaOMT2 (Table 1), they were insufficiently characterized and may have been erroneously annotated in the GenBank as COMT genes.

The "promiscuity" of plant COMTs for flavonoid substrates is not unusual and has been reported by several workers (e.g., Gauthier et al., 1998; Frick & Kutchan, 1999; Chiron et al., 2000; Ibdah et al., 2003). A well-documented example is that of *Chrysosplenium americanum* Schwein ex Hooker (Saxifragaceae) OMT1 and OMT2, which differ in three amino acids in their primary sequences, exhibit differential affinities toward phenylpropanoids such as caffeic and 5HF acids, and flavonoids such as quercetin and luteolin (Gauthier et al., 1998). This is partly because of the structural similarity between the phenylpropanoid moiety and the B-ring and 3-C side chain of flavonoids (Fig. 1), the high sequence homologies between COMTs and flavonoid OMTs, as well as the reportedly spaceous active site of COMT to accommodate the 3- and 5-substituted phenolic substrates (Zubieta et al., 2002). It has also been shown that a difference of as little as one or a few amino acids near the active site can change the substrate preferences of OMTs (Wang & Pichersky, 1998; Yang et al., 2004). It is imperative, therefore, that previously characterized proteins as COMTs be reexamined for their substrate preferences, coupled with a rigorous characterization of the enzyme reaction products, if erroneous annotation of new OMT genes is to be avoided.

Tricin as a multifunctional nutraceutical

There is an increasing body of evidence on the potential benefits of tricin for human health. These include its relaxant effect on smooth muscle of intestinal tissues, powerful antioxidant effect (Bichoff et al., 1964), potent antihistaminic activity (Kuwabara et al., 2003), and growth inhibition of human malignant breast tumor cells and colon cancer cells (Hudson et al., 2000; Cai et al., 2004). In addition, tricin was recently shown to interfere with murine gastrointestinal carcinogenesis and is considered safe for clinical development as a cancer chemopreventive agent (Verschoyle et al., 2006). An acylated tricin glycoside from sugarcane (*Saccharum officinarum* L., Gramineae) juice exhibits *in vitro* antiproliferative activity against several human cancer cell lines with a higher selectivity toward cells of breast-resistant NIC/ADR line (Duarte-Alameida et al., 2007).

These reports represent a small sample of the thousands of entries of a tricin search in Sci-Finder or similar search engines. This attests to the significance of tricin as a naturally occurring phytochemical with a potential use as a nutraceutical in edible cereal grain crops, which normally contain small amounts in the relevant tissues. The

application of metabolic engineering technology, using tissue-specific (endosperm) promoter-driven wheat flavone OMT gene via *Agrobacterium* or direct transformation methods, should be useful in the development of tricin-enriched cereal grain products.

Conclusions

These results indicate that there exists a flavone-specific OMT gene family in cereal grain plants as well as other monocot species, all of which accumulate variable amounts of tricin (Harborne, 1975). The gene products of this family use tricetin as their preferred substrate, although they also accept luteolin to a lower extent. It is interesting to note that tricin is the predominant enzyme reaction product of tricetin in wheat (Zhou et al., 2006a) and rice (Zhou et al., 2006b), as well as in maize and barley (this work). This suggests that the flavone-specific OMT gene family may be more widespread than previously thought in cereal grain plants as well as in other monocot species.

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